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Prospective Cohort Study of the Relative Abundance of Extended-Spectrum-Beta-Lactamase-Producing *Escherichia coli* in the Gut of Patients Admitted to Hospitals

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A total of 458 patients were prospectively included at hospital admission and screened for extended-spectrum-beta-lactamase-producing (ESBL) *Escherichia coli* carriage in 2007 and in 2010 to 2012. A 4-fold increase in ESBL carriage (3% to 12%), a 5-fold increase in numbers of community patients among ESBL carriers, and a higher number of multiple ESBL strains was found in the 2010 to 2012 period. ESBL *E. coli* represented the dominant *E. coli* strain (relative abundance, >50%) in 10/32 (31%) of ESBL carriers. This represents a major threat in terms of infectious risk and dissemination.

The epidemiology of *Escherichia coli* infections has recently been profoundly modified with the global emergence of strains resistant to third-generation cephalosporins (3GC), specifically, strains producing extended-spectrum β-lactamases (ESBL), in both hospitals and the community (1). CTX-M-type ESBLs, which have spread in a polyclonal manner on all continents, constitute a major public health concern worldwide (1).

Gut colonization is the first step toward infections by *E. coli* (2). Recently, it has been shown that a high relative abundance (RA) of ESBL *E. coli* in the feces increases the risk of urinary tract infection (UTI) by ESBL *E. coli* (3). Additionally, a high ESBL *E. coli* RA has been associated with longer fecal carriage time, increasing the numbers of excreted *E. coli* in the environment and the potential risk of dissemination of these resistant strains (4). However, most studies focusing on ESBL fecal carriage have described the presence or absence of ESBL *E. coli* in the gut but have lacked quantitative data. Moreover, whether the ESBL populations are dominant was not specified. Here, we characterized and determined the prevalence, bacterial load, and diversity of ESBL *E. coli* in the gut of patients at hospital admission at two different time periods and determined factors associated with high relative abundance of ESBL carriage.

We used samples collected in two previous prospective clinical trials (5, 6) (https://clinicaltrials.gov/ct2/show/NCT00520715 and https://clinicaltrials.gov/ct2/show/NCT01209247). In both works, clinical data (age, sex, Charlson comorbidity index, hospitalization ward, history of immunosuppression, previous hospitalization in the last 12 months, previous antibiotic exposure in the last 3 months, and antibiotic class) as well as a rectal swab had been collected from patients at admission in the same two tertiary-care hospitals from the Paris region in France (5, 6). The first collection period (P1) was between May and November 2007, and the second period (P2) was between October 2010 and July 2012. Both studies were approved by the local institutional review board (IRB 00008522), and patients gave informed consent (5, 6). Rectal swabs were discharged in 1 ml of brain heart infusion (BHI) broth with glycerol and stored at −80°C in duplicates (5, 6). After thawing, 50 μl was plated on Drigalski agar plates (Pasteur Diagnostics, Paris, France), one containing no antibiotics and one containing 1 μg/ml cefotaxime. Up to 10 colonies with distinct morphotypes growing on plates with or without cefotaxime were identified by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) analysis and tested for antibiotic susceptibility by the disk diffusion method. Presence of an ESBL was suspected after using the synergy test (7). After DNA extraction, phylogroups and ST131 clonal group were determined by PCR (8, 9). Genes encoding CTX-M, TEM, and SHV were amplified by PCR and sequenced (10). Inter- and intrapatient relatedness between ESBL *E. coli* strains were assessed by repetitive element palindromic PCR (rep-PCR) (3). Relative densities of total *E. coli* and of resistant *E. coli* were determined by plating serial dilutions of the broth onto Drigalski agar, with or without 1 μg/ml cefotaxime. The ESBL RA was calculated as the ratio of the ESBL *E. coli* counts divided by the total *E. coli* number, expressed as a percentage. *E. coli* clones were considered dominant if they represented 50% or more of the *E. coli* population (11).

We included 458 patients in this work: 260 during P1 and 198 during P2. P1 patients were older than P2 patients (average P1 age, 66.6 years [standard deviation, or SD, 18.9] versus 57 years [SD, 16.1] for P2 patients; $P < 0.01$); patient characteristics were otherwise similar in both periods. Table 1 represents the characteristics of ESBL *E. coli* strains carried in P1 and P2 patients. We found a 4-fold increase in the prevalence of ESBL *E. coli* carriage between P1 and P2 (8/260 [3.1%] versus 24/198 [12.1%]; $P < 0.01$), highlighting the major increase in ESBL dissemination in both the

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TABLE 1 Characteristics of ESBL *E. coli* strains in patients at admission in the two different time periods

Parameter	Period 1	Period 2	*P* value
Collection period (yr[s])	2007	2010–2012	
Total no. of patients	260	198	
No. of patients carrying ESBL *E. coli* (95% CI)	8 (1–5)	24 (15–33)	<0.01
No. of patients admitted from community (95% CI)	129 (111–143)	91 (77–104)	0.44
No. of community patients carrying ESBL *E. coli* (95% CI)	2 (0–3)	9 (11–27)	<0.001
No. of different ESBL *E. coli* isolates	10	40	
Median no. (range) of ESBL *E. coli* isolates/subject	1.25 (1–2)	2 (1–4)	<0.01
No. of subjects among ESBL carriers with >1 ESBL *E. coli* isolate (95% CI)	1 (0–3)	16 (11–21)	0.01
No. of isolates with ESBL type:			0.70
CTX-M-1	1	7	
CTX-M-15	6	22	
CTX-M-32	0	2	
CTX-M-14	2	3	
CTX-M-27	0	2	
TEM-52	1	1	
SHV-12	0	3	
No. of isolates in phylogroup:			0.17
A	5	10	
B1	2	12	
B2	3	5	
D	0	7	
F	0	6	
No. of isolates in ST131 clonal group	0	3	
Mean (SD) relative abundance (%) of ESBL *E. coli*	17 (34)	41 (42)	0.31

community and in hospitals in France, as elsewhere in the world. Nearly half the subjects (220/458) could be considered "community" patients, as they had not been hospitalized in the last year. While community patients remained a minority of ESBL carriers (Table 1), the ESBL carriage rate increased 6-fold (from 2/129 [1.5%] to 9/91 [9.8%]; $P < 0.001$) between P1 and P2 in the community patient population, suggesting wide dissemination of these isolates in the Parisian community, as previously described (12). Among the 32 ESBL carriers, 1/8 in P1 and 16/24 in P2 carried >1 genetically different ESBL *E. coli* isolate ($P < 0.01$), representing 10 different ESBL strains in P1 and 40 in P2. No clinical or microbiological factor was associated with the carriage of >1 distinct ESBL *E. coli* strain, apart from having been sampled in P2 ($P < 0.01$).

The investigation of resistance determinants found a predominance of CTX-M-15 enzymes both in P1 (6/10) and P2 (22/40; difference not statistically significant) and the presence of 6 other enzyme types. Concerning genetic background, no differences in phylogroup distribution could be found between the 2 collection periods, and only 3 subjects carried strains belonging to the ST131 clonal group, all of whom were in P2.

The median ESBL RA was 10% and ranged between 100% and 0.1%. In 10/32 (31%) ESBL carriers, ESBL *E. coli* represented the dominant strain (RA, >50%). Five subjects, all exposed to antibiotics in the previous 3 months, carried only ESBL *E. coli* in their gut (ESBL RA, 100%), including 2 patients who were not taking antibiotics at the time of study inclusion. The only clinical factor associated with a high ESBL RA was antibiotic exposure in the previous 3 months (mean, 35% [SD, 0.41%], versus 5% [SD, 0.04%] for subjects not exposed to antibiotics; $P = 0.01$) (Fig. 1). However, data concerning exposure to nonantibiotic treatments, such as protein pump inhibitors or, more importantly, concerning recent travel abroad, both of which have been found to be associated with ESBL carriage, were not collected (13).

The high bacterial load of ESBL in the gut constitutes a major threat both in terms of infectious risk for ESBL-colonized subjects as well as for the risk of dissemination to the environment and to other patients in hospitals. Although controversial, several works

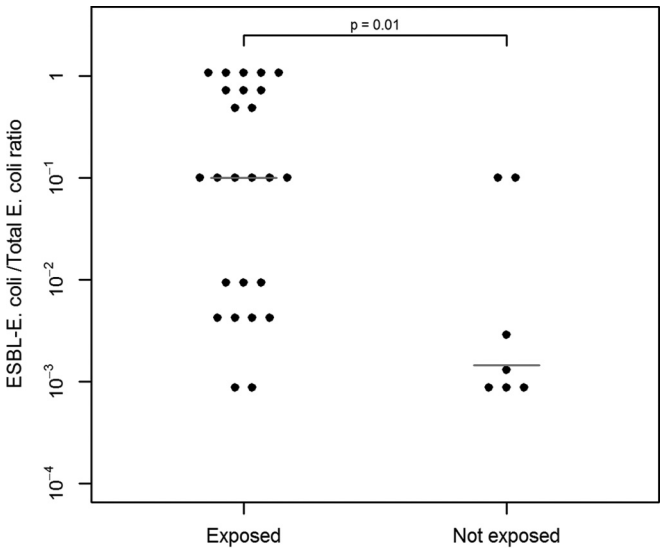

FIG 1 Relative abundance of *E. coli* ESBL in the gut of the 32 subjects who carried ESBL *E. coli*, according to previous antibiotic exposure.

have suggested that the RA of various *E. coli* clones present in the feces plays a key role in the pathogenesis of UTIs, probably because the dominant *E. coli* clone would have the maximum likelihood to colonize the urinary tract (14). Subsequently, a high RA of ESBL *E. coli* in the feces increases the risk of UTI by ESBL *E. coli* (3). Similarly, during bacterial translocation from the gut leading to bacteremia, the translocating bacteria, including *E. coli*, have been found to be mostly the dominant gut colonizer (15). Antibiotic exposure changes the respective proportions of gut *E. coli* populations and reduces the barrier effect, promoting the growth of resistant strains, as found here (16). As each day approximately 10^{10} *E. coli* per human are excreted into the environment, a high load of ESBL *E. coli* is likely to lead to dissemination, especially in a closed environment such as hospitals. Recently, a high RA of ESBL *E. coli* in feces was found to be associated with a longer carriage time, increasing yet again the burden of ESBL carriage (4).

Because the aim of this work was not to determine the specific ESBL types responsible for clinical infections, we cannot extrapolate that the ESBL types found in commensal *E. coli* are the same as those responsible for clinical infections. For instance, we found few strains belonging to the ST131 clonal group (3/28 among CTX-M-15 strains); this is the predominant *E. coli* ST lineage among extraintestinal pathogenic *E. coli* (ExPEC) isolates worldwide and belongs to the B2 phylogroup (17). Because we studied commensals, only 8 ESBL isolates were B2, and 3 among them were ST131. This may be seen as a study limitation; however, ESBL *E. coli* carriage has been shown in several studies to precede infection, and carriage of ESBL *E. coli* is linked to the risk of ESBL urinary tract infections in women with the same clone of ESBL *E. coli* found in urine samples as in fecal samples (3, 18, 19).

Altogether, these results emphasize the epidemic nature of ESBL *E. coli* colonization in France, as illustrated by the number of community patients colonized and the multiclonal aspect of the carriage. Additionally, the increase in strain diversity between the 2 study periods suggests a higher level of interindividual transmission and wide dissemination of these strains, a very worrying finding, especially because *E. coli* is the major human commensal bacterium. Limiting antibiotic consumption and interindividual transmission of resistant organisms remains the cornerstone of actions required to limit the spread of these multiresistant strains; yet, given the high abundance and enormous reservoir of *E. coli*, it may be too late.

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